

AMENDMENTS TO THE CLAIMS:

The following is the status of the claims of the above-captioned application, as amended.

Claim 1. (Currently amended) A 2 μ m-family plasmid comprising a polynucleotide sequence insertion, deletion and/or substitution between the a first base after the a last functional codon of at least one of either a an *REP* 2 gene or an *FLP* gene and the a last base before the an FRT site in an inverted repeat adjacent to said gene.

Claim 2. (Currently amended) The 2 μ m-family plasmid of Claim 1 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the *FLP* gene and/or the *REP* 2 gene has the sequence of a an *FLP* gene and/or a an *REP* 2 gene, respectively, derived from a naturally occurring 2 μ m-family plasmid.

Claim 3. (Currently amended) The 2 μ m-family plasmid of Claim 1, wherein the naturally occurring 2 μ m-family plasmid is selected from comprises pSR1, pSB3 or pSB4 as obtained from *Zygosaccharomyces rouxii*, pSB 1 from *Zygosaccharomyces bailli*, or pSB2 both as obtained from *Zygosaccharomyces bailli*, pSM1 as obtained from *Zygosaccharomyces fermentati*, pKD1 as obtained from *Kluyveromyces drosophilum*, pPM1 as obtained from *Pichia membranaefaciens*, and or the 2 μ m plasmid as obtained from *Saccharomyces cerevisiae*.

Claim 4. (Currently amended) The 2 μ m-family plasmid of Claim 2 wherein the sequence of the inverted repeat adjacent to said *FLP* and/or *REP* 2 gene is derived from the sequence of the corresponding inverted repeat in the same naturally occurring 2 μ m-family plasmid as the sequence from which the gene is derived from.

Claim 5. (Currently amended) The 2 μ m-family plasmid of ~~Claims~~ Claim 2 wherein the naturally occurring 2 μ m-family plasmid is the 2 μ m plasmid as obtained from *Saccharomyces cerevisiae*.

Claim 6. (Currently amended) The 2 μ m-family plasmid of Claim 5 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between the a first base of codon 59 of the *REP* 2 gene and the last base before the FRT site in the adjacent inverted repeat.

Claim 7. (Currently amended) The 2 μ m-family plasmid of Claim 5 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the sequence of the *REP2* gene and the adjacent inverted repeat ~~is as defined by SEQ ID NO: 1 or variant thereof.~~ comprises the nucleotides of SEQ ID NO: 1, or a nucleotide sequence 95% identical to SEQ ID NO:1.

Claim 8. (Currently amended) The 2 μ m-family plasmid of Claim 1 wherein polynucleotide sequence insertion, deletion and/or substitution occurs at a position between ~~the~~ a first base of the inverted repeat and ~~the~~ a last base before the FRT site.

Claim 9. (Currently amended) The 2 μ m-family plasmid of Claim 1 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs between ~~the~~ a first base after the end of the *REP2* coding sequence and the last base before the FRT site, ~~such as at the first base after the end of the *REP2* coding sequence.~~

Claim 10. (Currently amended) The 2 μ m-family plasmid of Claim 1 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the inverted repeat that follows the *REP2* coding sequence has a sequence ~~derived from the~~ a corresponding region of the 2 μ m plasmid ~~as obtained from *Saccharomyces cerevisiae* and preferably the polynucleotide sequence insertion, deletion and/or substitution occurs at an *XcmI* site or an *FspI* site within the inverted repeat.~~

Claim 11. (Currently amended) The 2 μ m-family plasmid of Claim 5 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between ~~the~~ a first base of codon 344 of the *FLP* gene and the last base before the FRT site in the adjacent inverted repeat.

Claim 12. (Currently amended) The 2 μ m-family plasmid of Claim 5 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the sequence of the *FLP* coding sequence and the adjacent inverted repeat comprises the nucleotides of SEQ ID NO: 2, or a nucleotide sequence 95% identical to SEQ ID NO:2. ~~is as defined by SEQ ID NO:2 or variant thereof.~~

Claim 13. (Currently amended) The 2 μ m-family plasmid of Claim 11 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between ~~the~~a first base of the inverted repeat and the last base before the FRT site.

Claim 14. (Currently amended) The 2 μ m-family plasmid of Claim 13 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at a position between ~~the~~a first base after the end of the *FLP* coding sequence and the last base before the FRT site.

Claim 15. (Currently amended) The 2 μ m-family plasmid of Claim 14 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at ~~the~~a first base after the end of the *FLP* coding sequence.

Claim 16. (Currently amended) The 2 μ m-family plasmid of Claim 11 wherein, other than the polynucleotide sequence insertion, deletion and/or substitution, the inverted repeat that follows the *FLP* gene has a sequence ~~derived from the~~a corresponding region of the 2 μ m plasmid as obtained from *Saccharomyces cerevisiae*, ~~and preferably the polynucleotide sequence insertion, deletion and/or substitution occurs at an *HgaI* site or an *FspI* site within the inverted repeat.~~

Claim 17. (Currently amended) The 2 μ m-family plasmid of Claim 1 comprising polynucleotide sequence insertions, deletions and/or substitutions between ~~the~~a first ~~bases~~base after the last functional codons of both of the *REP2* gene and the *FLP* gene and a last ~~bases~~base before the FRT sites in the inverted repeats adjacent to each of said genes, which polynucleotide sequence insertions, deletions and/or substitutions can be the same or different.

Claim 18. (Currently amended) The 2 μ m-family plasmid of Claim 1 ~~additionally,~~₁ comprising a polynucleotide sequence insertion, deletion and/or substitution which is not ~~at a position as defined in any one of the preceding claims~~between the first base and the last base.

Claim 19. (Original) The 2 μ m-family plasmid of Claim 18 wherein the polynucleotide sequence insertion, deletion and/or substitution occurs within an untranscribed region around an ARS sequence.

Claim 20. (Previously presented) The 2 μ m-family plasmid of Claim 1 wherein the, or at least one, polynucleotide sequence insertion, deletion and/or substitution is a polynucleotide sequence insertion.

Claim 21. (Original) The 2 μ m-family plasmid of Claim 20 in which the polynucleotide sequence insertion encodes an open reading frame.

Claim 22. (Original) The 2 μ m-family plasmid of Claim 21 in which the open reading frame encodes a non-2 μ m-family plasmid protein.

Claim 23. (Currently amended) The 2 μ m-family plasmid of Claim 22 in which the non-2 μ m-family plasmid protein comprises the sequence of a protein involved in protein folding, or which has chaperone activity or is involved in the unfolded protein response, albumin, a monoclonal antibody, an etoposide, a serum protein (~~such as a blood clotting factor~~), antistatin, a tick anticoagulant peptide, transferrin, lactoferrin, endostatin, angiostatin, collagens, immunoglobulins or immunoglobulin-based molecules or fragments of either (e.g. a dAb, Fab' fragments, F(ab')₂, scAb, scFv or scFv fragment), a Kunitz domain protein, interferons, interleukins, IL 10, IL 11, IL2, interferon α species and sub-species, interferon β species and sub-species, interferon γ species and subspecies, leptin, CNTF, CNTF_{AX15}, IL 1-receptor antagonist, erythropoietin (EPO) and EPO mimics, thrombopoietin (TPO) and TPO mimics, prosaptide, cyanovirin-N, 5-helix, T20 peptide, T1249 peptide, HIV gp41, HIV gp120, urokinase, prourokinase, tPA, hirudin, platelet derived growth factor, parathyroid hormone, proinsulin, insulin, glucagon, glucagon-like peptides, insulin-like growth factor, calcitonin, growth hormone, transforming growth factor β , tumour necrosis factor, G-CSF, GM-CSF, M-CSF, FGF, coagulation factors in both pre and active forms, including but not limited to plasminogen, fibrinogen, thrombin, pre-thrombin, pro-thrombin, von Willebrand's factor, ~~α_1 -antitrypsin~~ antitrypsin, plasminogen activators, Factor VII, Factor VIII, Factor IX, Factor X and Factor XIII, nerve growth factor, LACI, platelet-derived endothelial cell growth factor (PD-ECGF), glucose oxidase, serum cholinesterase, aprotinin, amyloid precursor protein, inter-alpha trypsin inhibitor, antithrombin III, apo-lipoprotein species, Protein C, or Protein S, ~~or a variant or fragment of any of the above.~~

Claim 24. (Currently amended) The 2 μ m-family plasmid of Claim 23 in which the 2 μ m-family plasmid protein comprises the sequence of albumin, ~~a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.~~

Claim 25. (Currently amended) The 2 μ m-family plasmid of Claim 23 in which the non-2 μ m-family plasmid protein comprises the sequence of transferrin, ~~a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.~~

Claim 26. (Currently amended) The 2 μ m-family plasmid of Claim 23 in which the non-2 μ m-family plasmid protein comprises the sequence of lactoferrin, ~~a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.~~

Claim 27. (Currently amended) The 2 μ m-family plasmid of Claim 23 in which the non-2 μ m-family plasmid protein comprises the sequence of Fc, ~~a variant or fragment thereof, or a fusion protein comprising the sequence of any of these.~~

Claim 28. (Original) The 2 μ m-family plasmid of Claim 23 in which the non-2 μ m-family plasmid protein comprises the sequence of a protein involved in protein folding, or which has chaperone activity or is involved in the unfolded protein response as encoded by any one of *AHA1*, *CCT2*, *CCT3*, *CCT4*, *CCT5*, *CCT6*, *CCT7*, *CCT8*, *CNS1*, *CPR3*, *CPR6*, *EPS1*, *ERO1*, *EUG1*, *FMO1*, *HCH1*, *HSP10*, *HSP12*, *SP104*, *HSP26*, *HSP30*, *HSP42*, *HSP60*, *HSP78*, *HSP82*, *JEM1*, *MDJ1*, *MDJ2*, *MPD1*, *MPD2*, *PD11*, *PFD1*, *ABC1*, *APJ1*, *ATP11*, *ATP12*, *BTT1*, *CDC37*, *CPR7*, *HSC82*, *KAR2*, *LHS1*, *MGE1*, *MRS11*, *NOB1*, *ECM10*, *SSA1*, *SSA2*, *SSA3*, *SSA4*, *SSC1*, *SSE2*, *SIL1*, *SLS1*, *UBI4*, *ORM1*, *ORM2*, *PER1*, *PTC2*, *PSE1* and *HAC1* or a truncated intronless *HAC1*.

Claim 29. (Previously presented) The 2 μ m-family plasmid of Claim 23 in which the chaperone is protein disulphide isomerase (PDI), or is a protein encoded by *ORM2*, *SSA1* or *PSE1*.

Claim 30. (Previously presented) The 2 μ m-family plasmid of Claim 22 in which the non-2 μ m-family plasmid protein comprises a secretion leader sequence.

Claim 31. (Original) The 2 μ m-family plasmid of Claim 22 in which the non-2 μ m-family plasmid protein comprises the sequence of a bacterial selectable marker and/or a yeast selectable marker.

Claim 32. (Original) The 2 μ m-family plasmid of Claim 31 in which the bacterial selectable marker is a β -lactamase gene and/or the yeast selectable marker is a *LEU2* selectable marker.

Claim 33. (Currently amended) The 2 μ m-family plasmid according to Claim 1, which plasmid comprises (i) a heterologous sequence encoding a non- 2 μ m-family plasmid protein; (ii) a heterologous sequence encoding a protein comprising the sequence of a protein involved in protein folding, a chaperone or a protein involved in the unfolded protein response, preferably protein disulphide isomerase; and (iii) a heterologous sequence encoding a protein comprising the sequence of a selectable marker; wherein at least one of the heterologous sequences occurs at a position as defined by any one of Claims 1 to 16, between the first base after the last functional codon of at least one of either the *REP2* gene or the *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to the gene.

Claim 34. (Currently amended) A method of preparing a plasmid as defined by Claim 1 comprising:

- (a) providing a plasmid comprising the sequence of a *REP 2* gene and the inverted repeat that follows the *REP2* gene, or a *FLP* gene and the inverted repeat that follows the *FLP* gene, in each case the inverted repeat comprising an FRT site;
- (b) providing a polynucleotide sequence and inserting the polynucleotide sequence into the plasmid at a position as defined in of Claim 1 between the first base after the last functional codon of at least one of either the *REP2* gene or the *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to the gene; and/or
- (c) deleting some or all of the nucleotide bases at the positions between the first base after the last functional codon of at least one of either the *REP2* gene or the *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to the gene of defined in Claim 1; and/or
- (d) substituting some or all of the nucleotide bases between the first base after the last functional codon of at least one of either the *REP2* gene or the *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to the gene ~~at the positions defined in Claim 1~~ with alternative nucleotide bases.

Claim 35. (Original) A plasmid obtainable by the method of Claim 34.

Claim 36. (Previously presented) A host cell comprising a plasmid as defined by Claim 1.

Claim 37. (Original) A host cell according to Claim 36 which is a yeast cell.

Claim 38. (Previously presented) A host cell according to Claim 36 in which the plasmid is stable as a multicopy plasmid.

Claim 39. (Currently amended) A host cell according to Claim 38 in which the plasmid comprises a polynucleotide sequence insertion, deletion and/or substitution between a first base after a last functional codon of at least one of either an *REP 2* gene or an *FLP* gene and a last base before an FRT site in an inverted repeat adjacent to said gene. is based on pSR1, pSB3 or pSB4 and the yeast cell is *Zygosaccharomyces rouxii*, the plasmid is based on pSB1 or pSB2 and the yeast cell is *Zygosaccharomyces bailli*, the plasmid is based on pSM1 and the yeast cell is *Zygosaccharomyces fermentati*, the plasmid is based on pKD1 and the yeast cell is *Kluyveromyces drosophilarum*, the plasmid is based on pPM1 and the yeast cell is *Pichia membranaefaciens* or the plasmid is based on the 2µm plasmid and the yeast cell is *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*.

Claim 40. (Currently amended) A host cell according to Claim 38 in which, if the plasmid contains, or is modified to contain, a selectable marker then stability, as measured by the loss of the marker, is at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9% or substantially 100% after 5 generations.

Claim 41. (Previously presented) A method of producing a protein comprising the steps of-

- (a) providing a plasmid as defined by Claim 1;
- (b) providing a suitable host cell;
- (c) transforming the host cell with the plasmid; and
- (d) culturing the transformed host cell in a culture medium;
- (e) thereby to produce the protein.

Claim 42. (Currently amended) A method of producing a protein comprising the steps of providing a host cell as defined by Claim 36 which host cell comprises a plasmid comprising a polynucleotide sequence insertion, deletion and/or substitution between the first base after the last functional codon of at least one of either a *REP 2* gene or an *FLP* gene and the last base before the FRT site in an inverted repeat adjacent to said gene as defined by Claim 4 and culturing the host cell in a culture medium thereby to produce the protein.

Claim 43. (Previously presented) A method according to Claim 41 further comprising the step of isolating the thus produced protein from the cultured host cell or the culture medium.

Claim 44. (Currently amended) A method according to Claim 43 further comprising the step of purifying the thus isolated protein ~~to a commercially acceptable level of purity.~~

Claim 45. (Original) A method according to Claim 44 further comprising the step of formulating the thus purified protein with a carrier or diluent, and optionally presenting the thus formulated protein in a unit form.

Claim 46. (Canceled)

Claim 47. (Currently amended) A method according to Claim 44 further comprising the step of formulating the ~~thus~~ purified protein with a pharmaceutically acceptable carrier or diluent and optionally presenting the thus formulated protein in a unit dosage form.

Claims 48 - 63. (Canceled).

Claim 64 (New) The 2 μ m-family plasmid of Claim 11, wherein the polynucleotide sequence insertion, deletion and/or substitution occurs at an *Hgal* site or an *Fspl* site within the inverted repeat.

Claim 65. (New) The 2 μ m-family plasmid of Claim 1, wherein the plasmid comprises a heterologous sequence encoding protein disulphide isomerase.

Claim 66 (New) The 2 μ m-family plasmid of Claim 1, wherein the plasmid comprises a heterologous sequence encoding a protein of interest.